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Facile synthesis of fused nitrogen containing heterocycles as anticancer agents

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Abstract

One pot three components reaction of the pyrazol-5-one **1** with different aromatic aldehydes and malononitrile was carried out in the presence of Ammonium acetate to furnish the corresponding pyrazolopyridines **2a-g**. Carrying out the same reactions in the presence of piperydine yielded the pyranopyrazoles **4a-c**. Facile formation of the pyrazolopyridopyrimidines **5a-c** and **6a,b** was occurred via boiling of the adducts **2a,b,e** in Formic acid or chloroacetic acid. Treatment of adduct **2a** with triethyl orthoformat yielded the pyrazolopyrrolopyridine derivative **7**. Compound **2a** was also reacted with DMFDMA to form the corresponding adduct **8**. Pyrazol-5-one **1** underwent three different component reactions using a variety of active methylene reagents. The utility ethylbenzoyl acetate and benzoyl acetonitrile yielded the products **9a-e** and **10** respectively. Using of ethylacetoacetate and ethyl cyano acetate reagents led to the formation of adducts **11** and **12a-c**, respectively. On the other hand, diethylmalonate and acetylacetone produced the corresponding products **13** and **14**. The bioactivity of the compounds (**2c, 2e, 2d, 4a, 4b, 9c, 9e, 11, 12b, 12c**) as antitumor agents against liver carcinoma was examined.

Keywords: Pyrazoles, Pyridopyrazoles, Pyrazolopyridopyrimidine, Pyranopyrazoles, Antihepatocellular Carcinoma (HCC).

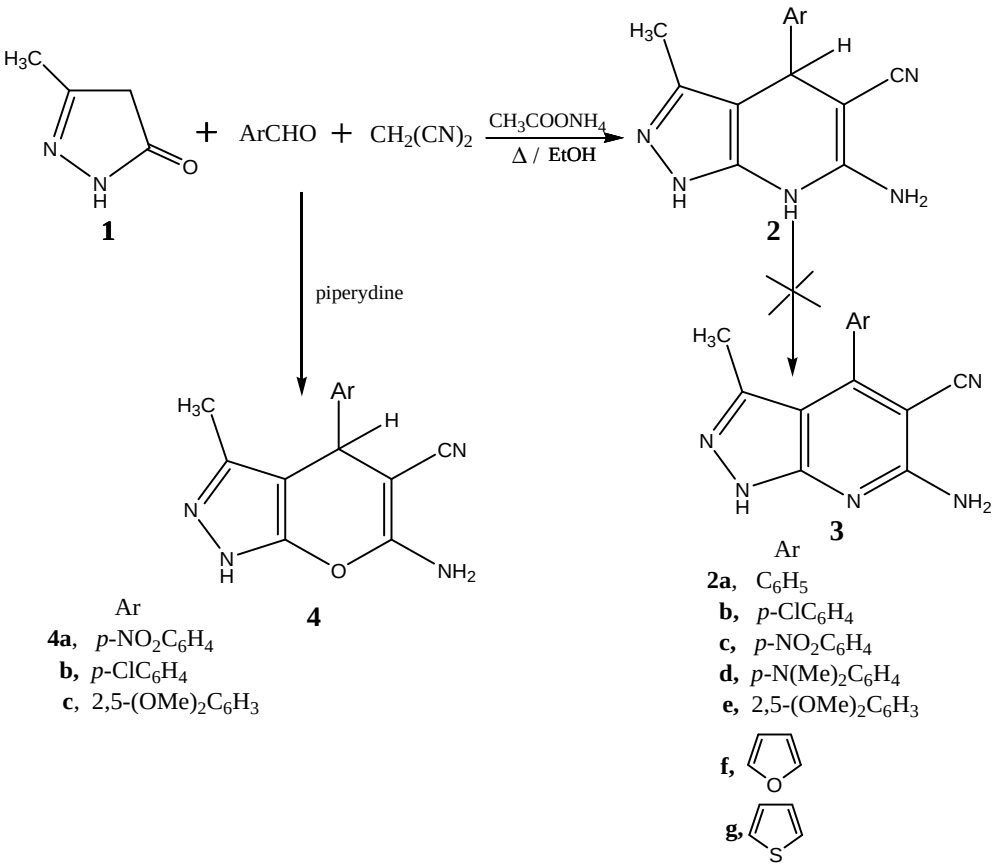
Introduction

Multicomponent reactions (MCRs) by virtue of their ease of execution and generally high yields of products have attracted considerable attention from the point of view organic synthesis[1-3]. Over the last few years, there has been a large development in the three-component reactions and great efforts to afford new and effective (MCRs)[4-6]. In addition, fused heterocycles systems like pyrazolopyridines, pyranopyrazoles and pyrazolopyrido - pyrimidines present interesting biological properties such as anticancer[7], fungicidal[8], bactericidal[9] and vasodilatory activities[10]. A considerable attention has been focused on the development of new methodologies to synthesize many kinds of these Nitrogen containing heterocycles[11,12]. Many of the previous multicomponent reactions for the

synthesis of pyrazolopyridopyrimidines were based on utilizing amino pyrazole or amino pyrimidine as starting materials with different catalysts[5,13]. Aiming to synthesize these important candidates with low cost starting materials and facile tools, we have prepared different fused derivatives *via* simple pyrazole (5-methyl-2,4-dihydro-3H-pyrazol-3-one) as starting material. Facile conversion of the pyrazolopyridines into pyrazolopyridopyrimidines has been reported. As for the multifocal nature of liver Carcinoma, the chemotherapy is the main choice for the liver cancer patient's treatment, so developing new therapeutic agents becomes an urgent need for liver cancer patients. Encouraged by this information and continuation to our previous work[14,15]. We have prepared in this report the formation of different derivatives of Nitrogen containing heterocycles and have evaluated their efficiency as an antihepatocellular *Carcinoma*.

Results and Discussion

A mixture of 5-methyl-2,4-dihydro-3H-pyrazol-3-one **1**, malononitrile and different aromatic aldehydes was heated in Ethanol containing Ammonium acetate under reflux to afford the corresponding 6-aminopyrazolo[3,4-b]pyridine-5-carbonitrile systems **2**. The reaction proceeded via the formation of the benzylidene adducts at first followed by the addition of malononitrile and cyclization *via* loss of water. It was explained previously that the formed adduct may undergo aromatization *via* loss of Hydrogen to produce structure **3[**16]. The ^{1}H-NMR spectrum of the all newly synthesized adducts revealed the presence of Hydrogen proton at C_4 at δ 4.6 ppm. This excluded structure **3** and supported the formation of the adduct **2**. Further more the X-ray of compound **2** was in accordance with the suggested structure (Scheme 1).



(Scheme 1)

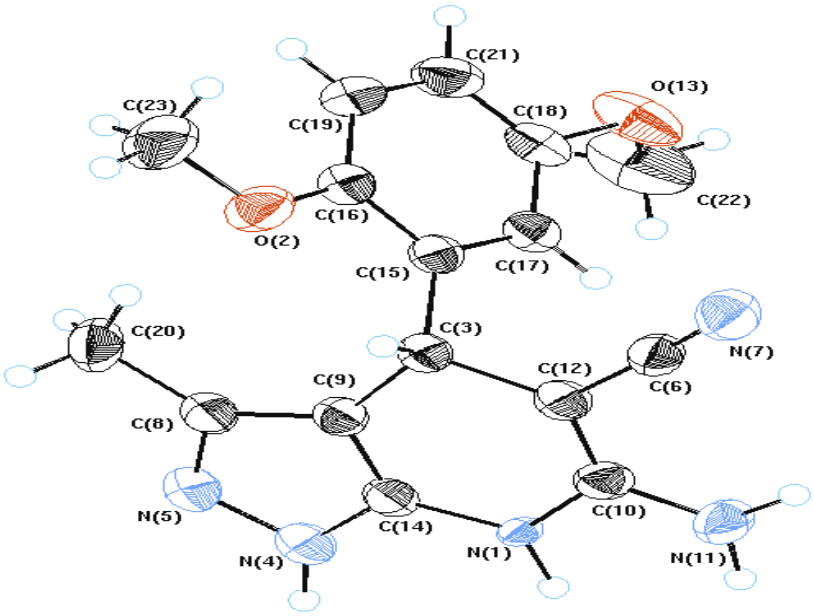


Figure 1

Molecular structure of the product **2** in the crystal; the crystallographic numbering doesn't represent the systematic numbering. Intermolecular bond lengths and bond angles. Selected bond lengths [Aº] and bond angles [º] limits use covalent radii + 0.020 Aº.

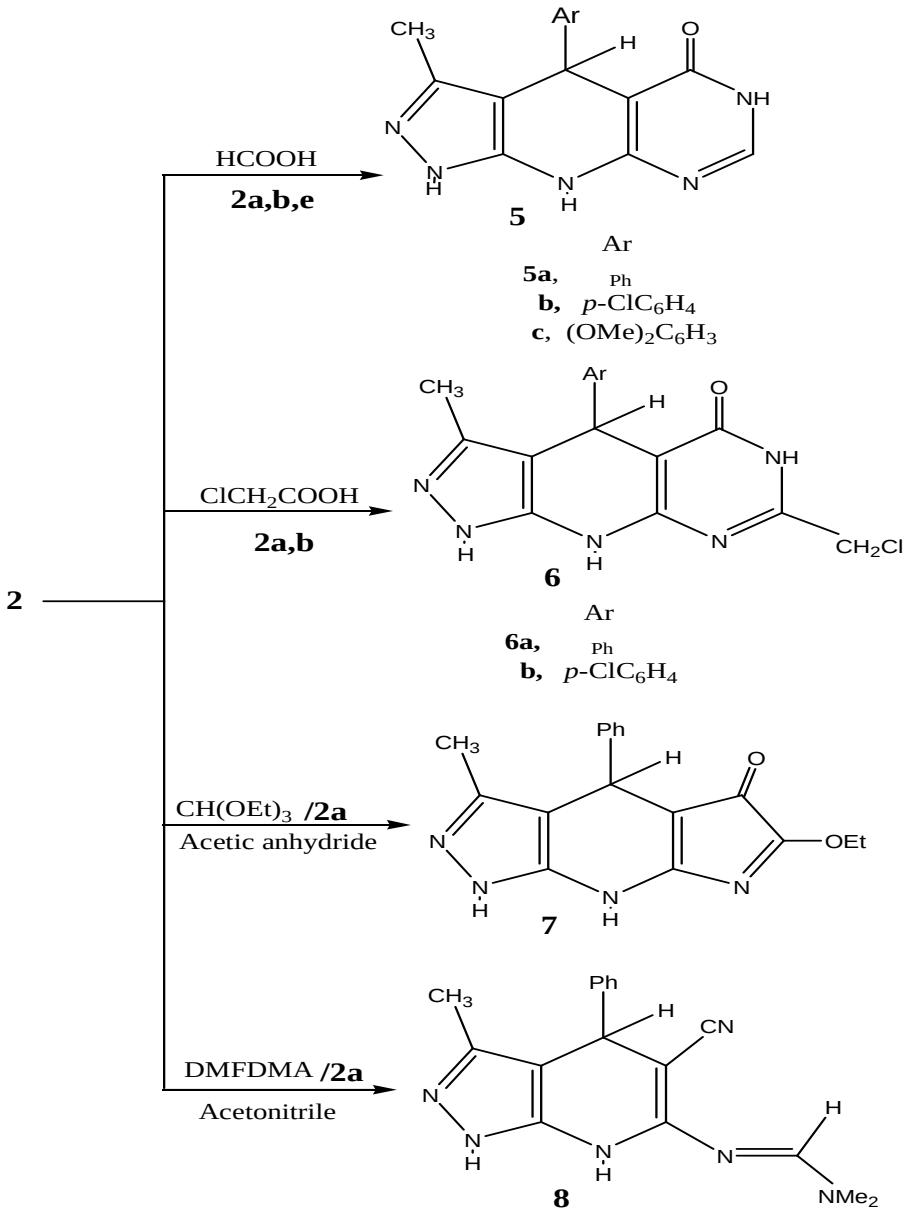
Bond length

[N(1)-C(10) 1.379(4)]; [N(1)-C(14) 1.377(4)]; [O(2)-C(16) 1.361(4)]; [O(2)-C(23)1.427(4)];[C(3)-C(9)1.504(4)];[C(3)-C(12)1.531(4)];[C(3)-C(15) 1.537(5)]; [N(4)-N(5) 1.367(4)]; [N(4)-C(14) 1.308(4)]; [N(5)-C(8) 1.344(4)]; [C(6)-N(7) 1.153(4)]; [C(6)-C(12)1.406(4)]; [C(8)-C(9)1.385(4)]; [C(8)-C(20) 1.499(5)]; [C(9)-C(14) 1.383(4)]; [C(10)-N(11)1.328(4)]; [C(10)-C(12) 1.367(4)]; [O(13) - C(18) 1.388(4)]; [O(13)-C(22) 1.363(5)]; [C(15)-C(16) 1.406(4)]; [C(15) - C(17) 1.388(4)]; [C(16)-C(19)1.387(5)];[C(17)-C(18)1.370(5)]; [C(18)-C(21) 1.378(5)]; [C(19)-C(21) 1.377(5)]; [N(1)- H(1) 0.960(2)]; [C(3)- H(3) 0.960(3)].

Bond Angles

[C(10)-N(1)-C(14) 115.8(2)]; [C(16)-O(2)-C(23) 118.2(3)]; [C(9)-C(3)-C(12) 106.8(2)]; [C(9)-C(3)-C(15) 112.2(3)]; [C(12)-C(3)-C(15) 112.6(3)]; [N(5)-N(4)-C(14) 102.2(3)]; [N(4)-N(5)-C(8) 112.9(3)]; [N(7)-C(6)-C(12) 177.1(3)]; [N(5)-C(8)-C(9) 106.7(3)]; [N(5)-C(8)-C(20) 120.9(3)]; [C(9)-C(8)-C(20)132.4(3)]; [C(3)-C(9)-C(8)134.2(3)];[C(3)-C(9)-C(14)122.8(3)];[C(8)-C(9)-C(14)103.0(3)]; [N(1)-C(10)-N(11) 109.7(3)]; [N(1)-C(10)-C(12) 122.5(3)]; [N(11)-C(10)-C(12) 127.8(3)]; [C(3)-C(12)-C(6) 116.4(3)]; [C(3)-C(12)-C(10) 126.0(3)];[C(6)-C(12)-C(10)117.6(3)]; [C(18)-O(13)-C(22)118.1(3)]; [N(1)-C(14)-N(4) 118.8(3)]; [N(1)- C(14)-C(9) 126.0(3)]; [N(4)-C(14)-C(9) 115.3(3)]; [C(3)-C(15)-C(16) 119.4(3)];[C(3)-C(15)-C(17)122.2(3)];[C(16)-C(15)-C(17) 118.4(3)]; [O(2)-C(16)-C(15) 116.2(3)]; [O(2)-C(16)-C(19) 123.9(3)]; [C(15)-C(16)-C(19) 119.9(3)]; [C(15)-C(17)-C(18) 121.6(3)]; [O(13)-C(18)-C(17) 122.5(3)]; [O(13)-C(18)-C(21) 118.0(3)]; [C(17)-C(18)-C(21) 119.4(3)]; [C(16)-C(19)-C(21) 119.9(3)]; [C(18)-C(21)-C(19) 120.8(3)]; [C(10)-N(1)-H(1) 119.7(2)]; [C(14)-N(1)-H(1)124.6(2)]; [C(9)-C(3)- H(3) 109.4(3)]; [C(12)-C(3)-H(3) 109.3(3)]; [C(15)-C(3)-H(3)106.6(3)].

Carrying out the previous reaction in the presence of piperydine led to the formation of the adducts 6-aminopyrano[2,3-c]pyrazole-5-carbonitrile **4a-c** (Scheme 1). The ^{1}H-NMR spectrum showed the disappearance of the characteristic signal of the NH group of pyridine ring. All the microanalytical and other spectroscopic data were in accordance with the pyranopyrazole structure **4** (c.f. the experimental section).

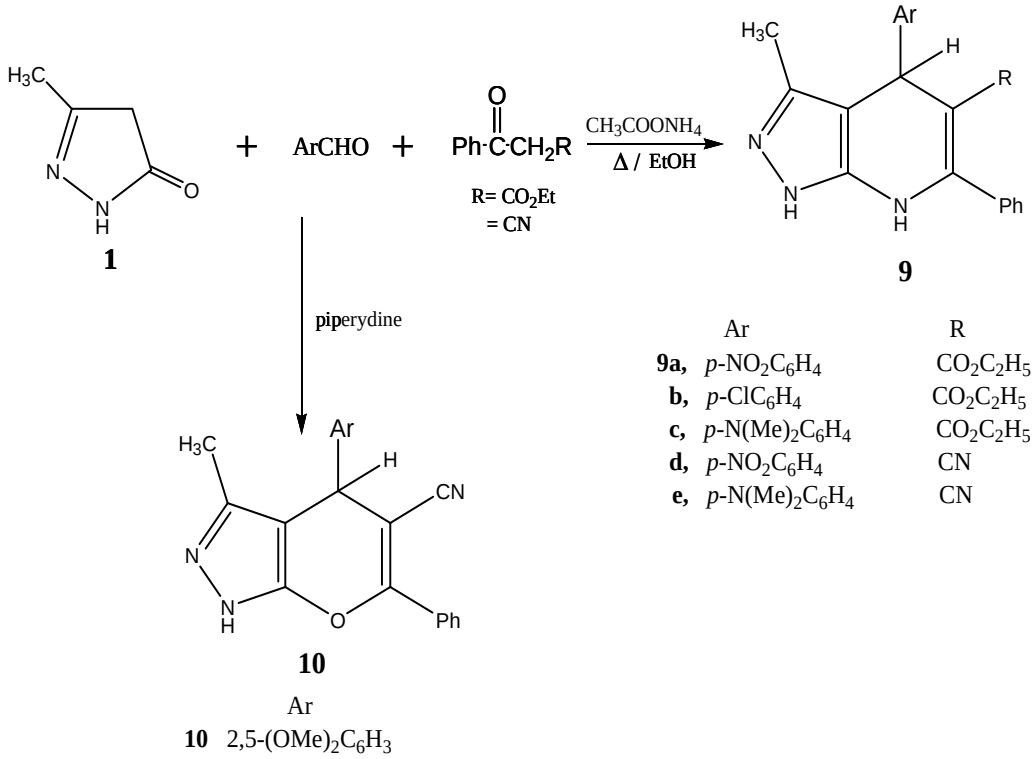


(Scheme2)

The pyrazolopyridopyrimidines **5a-c** and **6a,b** were formed easily *via* boiling of the pyrazolopyridines **2a,b,e** in organic acids e.g. Formic acid or Chloroacetic acid . It is believed that the nitrile group was converted at first into that amide group that was followed by cyclization. Dimorth rearrangement took place to furnish the final fused pyrazolopyrido-pyrimidine[17-18]. Treatment of the pyrazolopyridine **2a** with triethylor-thoformate in Acetic anhydride yielded the corresponding pyrazolopyrro-lopyridine adducts **7** (Scheme 2). The IR spectrum revealed the pyrrole carbonyl band at ν 1738 cm^{-1} and the ^{1}H-NMR showed the characteristic ethyl protons signals at δ 1.23 (t, 3H, CH_3); 4.30 (q, 2H, CH_2) ppm., besides the other signals that elucidate the suggested structure (c.f. the experimental section). In addition,

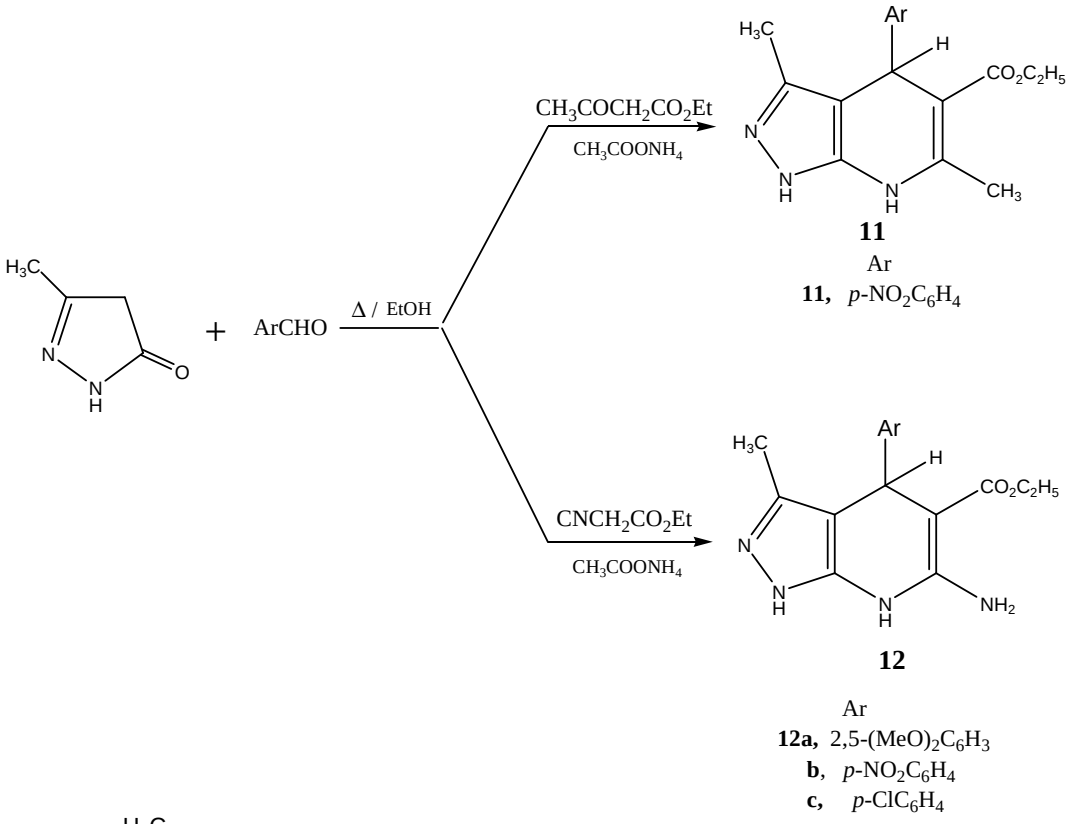
compound **2a** was allowed to react with DMFDMA in acetonitrile to form the open structure **8** (Scheme 2). ^{1}H-NMR confirmed the structure by revealing the signal corresponding to the sp^2 CH at δ 8.32 ppm and the singlet signal of the Me_2N at δ 3.13 ppm. The IR spectrum proved the presence of CN group band at ν 2188 cm^{-1} that supported the suggested structure (c.f. the experimental section).

In order to synthesize different types of pyrazolopyridine and pyranopy-razole; different active methylene reagents were utilized. The use of ethylbenzoylacetate and benzoylacetonitrile under the same previous conditions furnished the corresponding 6-phenylpyrazolo[3,4-b]pyridine-5-carboxylate **9** and 6-phenyl-pyrano[2,3-c]pyrazole-5-carbonitrile **10** (Scheme 3). The ^{1}H-NMR of **9a** taken as an example revealed the presence of a triplet at δ 1.03 ppm and quartet at δ 3.56 ppm characteristic of the ethyl ester protons. In addition, the appearance of the other signals characteristic of the aromatic protons at δ 7.37-8.41 ppm and NH exchangeable singlet signal at δ 12.15 ppm were present. On the other hand the IR spectrum showed the carbonyl ester band at ν 1728 cm^{-1} and the characteristic band of the CN group present in **9e** at ν 2221 cm^{-1} (c.f. the experimental section).



(Scheme 3)

Similarly, we examined the three component reaction of the pyrazolone **1** with the use of ethylacetoacetate and ethylcyanoacetate. In this case the reaction yielded the corresponding pyrazolopyridine adducts **11** and **12a-c** (Scheme 4). In addition, the use of diethylmalonate and acetylacetone produced the adducts **13, 14,** respectively (Scheme 4). Elucidation of the structure of all synthesized compounds was based on the microanalytical and the spectroscopic data.



11

Ar

11, *p*-$NO_2C_6H_4$

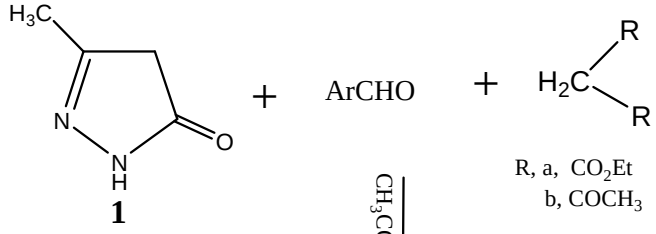
12

Ar

12a, 2,5-$(MeO)_2C_6H_3$

b, *p*-$NO_2C_6H_4$

c, *p*-ClC_6H_4



1

13

14

Ar

13, *p*-$NO_2C_6H_4$

14, $(OMe)_2C_6H_3$

(Scheme 4)

Antitumor activity

Evaluation of the anticancer activity of compounds (**2c, 2e, 2d, 4a, 4b, 9c, 9e, 11,12b, 12c**) was performed at the National Research Centre Institute (NRCI). The tested compounds were evaluated for thier cytotoxicity against the liver *Carcinoma* cell line (HEPG2) of human. Different concentrations of the tested compounds were added to the cell monolayer of the tumor. A 48 hours continuous drug exposure was used to estimate all the availability or growth. The cytotoxic activity of each compound was deduced from the dose response curves. Table (1) represents the cytotoxic activity for each compound.

Table (1): The cytotoxic activity

Sample Numbers	LC_{50} % at (µg/ml)
2c	70
2e	14
2d	49.7
4a	56
4b	49
9c	52.3
9e	66.6
11	0
12b	37.7
12c	78.4

Positive control LC_{50} (µg/ml) at: 2,
DMSO control = 3% at 100 (µg/ml), Negative control = 0% at 100 (µg/ml).

Materials and Methods

Synthetic methods, analytical and spectral data

Melting points were determined on an electrothermal apparatus (Buchi 535, Switzerland) in an open capillary tube and are uncorrected. IR spectra expressed in (cm^{-1}) were recorded in KBr pellets on a PA-9721 IR spectrophotometer. ^{1}H-NMR & ^{13}C-NMR spectra were obtained on a Varian EM-390 (270 and 500 MHz) spectrometer in DMSO-d_6 as solvent, using TMS as internal reference and chemical shifts (δ) are expressed in ppm. Mass spectra were recorded on Kratos (75e-v) Ms Equipment. Elemental analyses were carried out by the microanalytical unit at the National Research Centre, Giza, Egypt.

Synthesis of 6-amino-3-methyl-4-aryl-pyrazolo[3,4-b]pyridine-5-carbonitrile 2a-h. 6-amino-3-methyl-4-aryl-pyrano[3,4-b]pyridine-5-carbonitrile 4a-c.

General Procedure: A solution of pyrazolone **1** (0.98 g, 0.01 mol), arylaldehyde (0.01 mol) and malononitrile (0.66 g, 0.01 mol) in ethanol (10 ml) containing Ammonium acetate (0.98 gm, 2% excess) or piperidine (1 ml) was heated under reflux for 5 hr. The solvent was evaporated under vacuum and the remaining solids were treated with the proper solvent for crystallization.

6-Amino-3-methyl-4-phenyl-4,7-dihydro-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile ***2a.***

White crystals, mp. 240-242 ºC. (EtOH). (Yield, 89%); Ms (m/z, %): 251 (M^+, 19%). IR (KBr), ν cm^{-1}: 3410-3304 (NH_2, NH); 3001-2916 (CH_3); 2187 (CN); 1515 (C=C, aromatic). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): 1.70 (s, 3H, CH_3); 4.52 (s, 1H, CH); 6.82 (s, 2H, NH_2); 7.08-7.24 (m, 6H, 5 aromatic protons, NH); 12.04 (s, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-d_6), δ 9.78 (CH_3); 35.91 (C4); 56.06 (C5); 94.85 (C8); 104.79, 112.30, 113.21,

114.85, 127.90, 131.20 (aromatic carbons); 121.22 (C≡N); 150.72 (C3); 153.23 (C9); 159.20 (C6). Anal. Calcd. for $C_{14}H_{13}N_5$: C, 66.92%; H, 5.21% ; N, 27.87%. Found: C, 66.82%; H, 5.00%; N, 27.77%.

6-Amino-4-(4-chlorophenyl)-3-methyl-4-phenyl-4,7-dihydro-1H-pyrazolo-[3,4-b]pyridine-5-carbonitrile ***2b.***
Pale yellow crystals mp. 285 ºC. (EtOH). (Yield, 75%); Ms (m/z, %): 285 (M^+, 5%); 175 (M^+-PhCl, 100%). IR (KBr). ν cm^{-1}: 3407-3306 (NH_2); 3174-2916 (C-H, aliphatic); 2185 (CN); 1400 (C-Cl). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): 1.75 (s, 3H, CH_3); 4.59 (s, 1H, CH); 6.89 (s, 2H, NH_2); 7.16-7.34 (m, 5H, aromatic protons, NH); 12.04 (s, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-d_6), δ 9.35 (CH_3); 33.93 (C4); 55.86 (C5); 96.80 (C8); 120.58 (CN); 122.94, 125.13, 127.89, 128.06, 134.94, 145.42 (aromatic carbons); 151.13 (C3); 153.71 (C9); 160.18 (C6). Anal. Calcd. For $C_{14}H_{12}ClN_5$: C, 58.85%; H, 4.23%; Cl, 12.41%; N, 24.51%. Found: C, 58.76%; H, 4.03%; Cl, 12.31%; N, 24.14%.

6-Amino-4-(4-nitrophenyl)-3-methyl-4-phenyl-4,7-dihydro-1H-pyrazolo-[3,4-b]pyridine-5-carbonitrile ***2c.***
Yellow crystals mp. 276-277 ºC. (EtOH). (Yield, 95%); Ms (m/z, %): 296 (M^+, 1%); 280 (M^+-NH_2, 9%). IR (KBr). ν cm^{-1}: 3450 (NH); 3330 (NH_2); 3090 (CH, aromatic); 2965 (CH_3); 2210 (C≡N); 1605 (NO_2). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): 2.10 (s, 3H, CH_3); 4.98 (s, 1H, CH); 7.10 (s, 2H, NH_2) 7.37-7.40 (d, 2H, aromatic protons); ; 8.11-8.10 (m, 3H, 2 aromatic protons and NH); 11.41 (br, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-d_6), δ 9.70 (CH_3); 35.45 (C4); 55.50 (C5); 96.45 (C8); 121.53 (CN); 124.94, 125.13, 129.89, 130.06, 136.94, 147.42 (aromatic carbons); 153.13 (C3); 155.71 (C9); 160.10 (C6). Anal. Calcd. For $C_{14}H_{12}N_6O_2$: C, 58.85%; H, 4.23%; Cl, 12.41%; N, 24.51%. Found: C, 58.70%; H, 4.11%; Cl, 12.21%; N, 24.30%.

6-Amino-4-(4-dimethylaminophenyl)-3-methyl-4-phenyl-4,7-dihydro-1H-pyrazolo[3,4-b] pyri -dine-5-carbonitrile ***2d.***
Yellow crystals mp. 235-236 ºC. (EtOH). (Yield, 86%); Ms (m/z, %): 294 (M^+, 21%); 251 (M^+-NMe_2, 4%). IR (KBr). ν cm^{-1}: 3695 (NH); 3385 (NH_2); 3100 (CH, aromatic); 2921 (CH_3); 2193 (CN); 1514 (C=C, aromatic). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): 1.71 (s, 3H, CH_3); 2.78 (s, 6H, $2CH_3$); 4.38 (s, 1H, CH); 6.50 (s, 2H, NH_2); 6.60-6.90 (m, 5H, 4aromatic protons and NH); 11.97 (s, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-d_6), δ 9.30 (CH_3); 31.50 (C4); 40.10 & 40.55 (NMe_2) 59.96 (C5); 96.60 (C8); 120.53 (CN); 123.94, 124.13, 128.89, 129.26, 135.89, 146.21 (aromatic carbons); 152.90 (C3); 155.70 (C9); 160.10 (C6). Anal. Calcd. For $C_{16}H_{18}N_6$: C 65.29%; H, 6.16%; N, 28.55%. Found: C, 65.11%; H, 6.00%; N, 28.40%.

6-Amino-4-(2,5-dimethoxyphenyl)-3-methyl-4-phenyl-4,7-dihydro-1H-pyrazolo-[3,4-b]pyri -dine-5-carbonitrile ***2e.***
Yellow crystals mp. 238 ºC. (EtOH). (Yield, 91%); Ms (m/z, %): 311 (M^+, 100%). IR (KBr). ν cm^{-1}: 3353 (NH); 3172 (NH_2); 3172 (CH, aromatic); 2927 (CH_3); 2198 (CN); 1520 (C=C, aromatic). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): δ1.81 (s, 3H, CH_3); 3.64, 3.72 (2s, 6H, $2OCH_3$); 4.91 (s, 1H, CH); 6.52 (s, 2H, NH_2); 6.76-6.96 (m, 4H, 3aromatic protons and NH); 12.04 (s, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-d_6), δ 9.43 (CH_3); 34.42 (C4); 55.06, 55.98 ($2OCH_3$); 56.06 (C5); 97.58 (C8); 120.85 (C≡N); 111.60, 112.45, 113.20, 114.80, 133.32, 135.04 (aromatic carbons); 150.55 (C3); 155.23 (C9); 161.43 (C6). Anal. Calcd. For $C_{16}H_{17}N_5O_2$: C, 61.72%; H, 5.50%; N, 22.49%. Found: C, 61.59%; H, 5.45%; N, 22.36%.

6-Amino-4-furan-2-yl-3-methyl-4,7-dihydro-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile ***2f.***
Dark red crystals mp. 238 ºC. (EtOH). (Yield, 95%); Ms (m/z, %): 241 (M^+, 27%); 255 (M^+-NH_2, 26%). IR (KBr). ν cm^{-1}: 3589 (NH); 3353 (NH_2); 3170 (CH, aromatic); 2926 (CH_3); 2189 (CN); 1545 (C=C, aromatic). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): 2.00 (s, 3H, CH_3); 4.80 (s, 1H, CH); 6.21 (s, 2H, NH_2); 6.39-7.56 (m, 4H, Furan ring, NH); 12.19 (s, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-d_6), δ 9.35 (CH_3); 33.93 (C4); 59.96 (C5); 99.33 (C8); 120.23 (CN); 105.94, 122.13, 141.89, 152.95 (furan carbons); 155.13 (C3); 156.71 (C9); 160.18 (C6). Anal. Calcd. For $C_{12}H_{11}N_5O$: C, 59.74%; H, 4.60%; N, 29.03%. Found: C, 59.60%; H, 4.45%; N, 28.91%.

6-Amino-3-methyl-4-thiophen-2-yl-4,7-dihydro-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile ***2g.***
White crystals mp. 230 ºC. (EtOH). (Yield, 90%); Ms (m/z, %): 257 (M^+, 8%); 241 (M^+-NH_2, 5%). IR (KBr). ν cm^{-1}: 3539 (NH); 3357 (NH_2); 3168(CH, aromatic); 2925 (CH_3); 2188 (CN). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): 1.88 (s, 3H, CH_3); 4.95 (s, 1H, CH_3); 6.90 (s, 2H, NH_2), 7.00-7.34 (m, 4H, thiophene protons, NH); 12.13 (s, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-d_6), δ 9.35 (CH_3); 35.33 (C4); 59.86 (C5); 100.33 (C8); 120.23 (CN); 125.50, 128.3, 130.53, 150.42 (thiophene carbons); 156.13 (C3); 157.71 (C9); 160.18 (C6). Anal. Calcd. For $C_{12}H_{11}N_5S$: C, 56.01%; H, 4.31%; N, 27.22%. Found: C(55.93%) H, 4.20%; N, 27.00%.

6-Amino-3-methyl-4-(4-nitrophenyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile ***4a.***
Red crystals mp. 277-278 ºC. (Toluene). (Yield, 89%); Ms (m/z, %): 297 (M^+, 50%); 175 (M^+-$PhNO_2$, 100%). IR (KBr). ν cm^{-1}: 3330 (NH_2); 3132 (CH, aromatic); 2965 (CH_3); 2210 (C≡N); 1605 (NO_2). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): 1.73 (s, 3H, CH_3); 4.76 (s, 1H, CH); 7.06 (s, 2H, NH_2), 7.46-8.23 (m, 4H, 4 aromatic protons); 12.15 (s, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-d_6), δ 9.78 (CH_3); 34.93 (C4); 59.96 (C5); 96.60 (C8); 120.53 (CN); 123.94, 124.13, 128.89, 129.06, 135.94, 146.42 (aromatic carbons); 149.13 (C9); 150.71 (C3); 161.18 (C6). Anal. Calcd. For $C_{14}H_{11}N_5O_3$: C, 56.56%; H, 3.73%; N, 23.56%. Found: C, 56.40%; H, 3.53%; N, 23.40%.

6-amino-4-(4-chlorophenyl)-3-methyl-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile ***4b***
White crystals mp. 286-287 ºC. (EtOH). (Yield, 80%); Ms (m/z, %): 286 (M^+, 16.32%); 175 (M^+-PhCl, 100%). IR (KBr). ν cm^{-1}: 3307 (NH_2); 3104 (CH, aromatic); 2874 (C-H, Aliphatic); 2188 (C≡N). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): 1.79 (s, 3H, CH_3); 4.63 (s, 1H, CH); 6.93 (s, 2H, NH_2), 7.18-7.39 (m, 4H, 4 aromatic protons); 12.14 (s, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-d_6), δ 9.35 (CH_3); 34.03 (C4); 59.40 (C5); 96.65 (C8); 121.53 (CN); 124.94, 125.13, 129.89, 131.06, 135.90, 150.42 (aromatic carbons); 152.10 (C9); 154.61 (C3); 161.10 (C6). Anal. Calcd. For $C_{14}H_{11}ClN_4O$: C, 58.65%; H, 3.87%; Cl, 12.37%; N, 19.54%. Found: C, 58.55%; H, 3.67%; Cl, 12.17%; N, 19.24%.

6-Amino-4-(2,5-dimethoxyphenyl)-3-methyl-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile ***4c.***
White crystals mp. 246-248 ºC, (EtOH). (Yield, 76%); Ms (m/z, %): 312 (M^+, 55%). IR (KBr). ν cm^{-1}: 3420 (NH_2); 3104 (CH, aromatic); 2874 (C-H, Aliphatic); 2189 (C≡N), 1541 (C=C, aromatic). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): 2.12 (s, 3H, CH_3); 3.76, 3.87 (2s, 6H, $2OCH_3$); 5.02 (s, 1H, CH); 6.52 (s, 2H, NH_2); 6.77-7.63 (m, 3H, 3aromatic protons); 12.04 (s, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-d_6), δ 9.47 (CH_3); 34.03 (C4); 55.16, 55.89 ($2OCH_3$); 60.26 (C5); 96.80 (C8); 121.53 (CN); 123.90, 124.03, 128.78, 129.16, 136.94,

146.42 (aromatic carbons); 150.13 (C9); 155.71 (C3); 162.18 (C6). Anal. Calcd. For $C_{16}H_{16}N_4O_3$: C, 61.53%; H, 5.16%; N, 17.94%. Found: C, 61.33%; H, 5.00%; N, 17.74%.

Synthesis of 3-methyl-4-aryl-1,4,6,9-tetrahydro-pyrazolo[4',3':5,6]-pyrido[2,3-d]pyrimidin-5-one systems 5a-c.

General Procedure: A suspension of **2a, 2b or 2e** (0.01 mol) in Formic acid (5 ml) were heated under reflux for 6 hr. The solvent was evaporated under vacuum and the remaining residue was treated with ice water and neutralized by KOH (10%) and the precipitate was crystallized.

3-methyl-4-phenyl-1,4,6,9-tetrahydro-pyrazolo[4',3':5,6]pyrido[2,3-d]pyrimidin-5-one ***5a.***

White crystals mp. 176-177 ºC, Toluene. (Yield, 53%); Ms (m/z, %): 279 (M^+, 3%); 264 (M^+-Me, 2%). IR (KBr). ν cm^{-1}: 3097-3021 (CH, aromatic); 1680 (C=O); 1513 (C=C). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): 1.98 (s, 3H, CH_3); 4.09 (s, 1H, NH); 4.15 (s, 1H, CH); 7.14 (s, 1H, CH); 7.20-7.27 (m, 5H, aromatic protons); 8.2 (s, 1H, NH); 13.15 (s, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-d_6), δ 9.53 (CH_3); 35.60 (C4); 105.90 (C12); 107.03 (C10); 130.23, 132.05, 133.22, 135.23, 137.25, 138.26 (aromatic carbons); 150.21 (C3); 156.12 (C11); 160.23 (C13); 165.23 (C7); 168.06 (C=O). Anal. Calcd. For $C_{15}H_{13}N_5O$: C, 64.51%; H, 4.69%; N, 25.07%. Found: C, 64.31%; H, 4.49%; N, 24.93%.

4-(2,5-dimethoxyphenyl)-3-methyl-1,4,6,9-tetrahydro-pyrazolo-[4',3':5,6]pyrido[2,3-d]pyri - midin-5-one ***5b.***

White crystals mp. 176-177 ºC, (EtOH). (Yield, 69%); Ms (m/z, %): 339 (M^+, 46.5%). IR (KBr). ν cm^{-1}: 3110-3021 (CH, aromatic); 1685 (C=O); 1510 (C=C). 1H-NMR (270 MHz, DMSO-d_6, TMS): 2.06 (s, 3H, CH_3); 3.65,3.75 (2s, 6H, $2OCH_3$); 4.52 (s, 1H, CH); 6.66-6.84 (m, 4H, aromatic protons and NH); 6.94 (s, 1H, CH); 8.94 (s, 1H, NH); 13.85 (s, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-d_6), 9.58 (CH_3); 35.60 (C4); 49.23 & 51.23 ($2OCH_3$); 103.90 (C12); 105.03 (C10); 112.23, 115.05, 116.22, 125.23, 157.25, 160.26 (aromatic carbons); 149.35 (C3); 157.02 (C11); 158.20 (C13, pyridine); 163.23 (C7); 168.03 (C=O). Anal. Calcd. For $C_{17}H_{17}N_5O_3$: C, 60.17%; H, 5.05%; N, 20.64%. Found: C, 59.96%; H, 4.90%; N, 20.43%.

4-(4-chlorophenyl)-3-methyl-1,4,6,9-tetrahydro-pyrazolo[4',3':5,6]-pyrido[2,3-d]pyrimidin-5-one ***5c.***

White crystals mp. 236-237 ºC, (EtOH). (Yield, 59%); Ms (m/z, %): 313 (M^+, 50%). IR (KBr). ν cm^{-1}: 3100-3001 (CH, aromatic); 1680 (C=O); 1510 (C=C). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): 2.05 (s, 3H, CH_3); 4.17 (s, 1H, CH); 6.55-7.02 (m, 5H, aromatic protons and NH); 7.31 (s, 1H, CH); 9.10 (s, 1H, NH); 13.85 (s, 1H, NH).^{13}C-NMR (270 MHz, DMSO-d_6), δ 9.58 (CH_3); 33.60 (C4); 104.90 (C12); 107.03 (C10); 128.23, 129.05, 133.22, 136.23, 137.25, 138.26 (aromatic carbons); 149.35 (C3); 154.02 (C11); 159.20 (C13, pyridine); 163.23 (C7); 168.03 (C=O). Anal. Calcd. For $C_{15}H_{12}ClN_5O$: C, 57.42%; H, 3.86%; Cl, 11.30%; N, 22.32%. Found: C, 57.30%; H, 3.75%; Cl, 11.10%; N, 22.00%.

Synthesis of 7-(chloromethyl)-3-methyl-4-aryl-1,4,6,9-tetrahydro-5H-pyrazolo[4',3':5,6] pyrido [2,3-d]pyrimidin-5-one systems ***6a,b.***

General Procedure: A solution of **2a, 2b** (0.01 mol) in DMF (10 ml) and chloroacetic acid (0.94 g, 0.01mol) were heated under reflux for 7 h. The solution was evaporated under vacuum and the remaining solids were treated with the proper solvent for crystallization.

7-(chloromethyl)-3-methyl-4-phenyl-1,4,6,9-tetrahydro-pyrazolo-[4',3':5,6]pyrido[2,3-d]pyrimidin-5-one ***6a.***

Pale brown crystals mp. 161-162 ºC, (EtOH). (Yield, 40%); Ms (m/z, %): 327 (M^+, 25%). IR (KBr). ν cm^{-1}: 3001 (CH, aromatic); 2985 (CH, aliphatic); 1665 (C=O); 1510 (C=C). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): 1.89 (s, 3H, CH_3); 3.17 (s, 2H, CH_2); 4.93 (s, 1H, CH); 7.05 (s, 1H, NH); 7.40-7.60 (m, 5H, aromatic protons); 7.91 (s, 1H, NH); 12.03 (s, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-d_6), 9.58 (CH_3); 36.60 (C4); 52.06 (CH_2Cl); 103.90 (C12); 105.03 (C10); 125.23, 128.05, 130.22, 132.23, 135.25, 137.26 (aromatic carbons); 150.35 (C3); 155.02 (C11); 159.20 (C13); 164.23 (C7); 168.03 (C=O). Anal. Calcd. For $C_{16}H_{14}ClN_5O$: C, 58.63%; H, 4.31%; Cl, 10.82%; N, 21.37%. Found: C, 58.50%; H, 4.11%; Cl, 10.52%; N, 21.07%.

7-(chloromethyl)-4-(4-chlorophenyl)-3-methyl-1,4,6,9-tetrahydro-pyrazolo[4',3':5,6]pyrido[2,3-d]pyrimidin-5-one ***6b.***

Brown crystals mp. 176-178 ºC, (EtOH). (Yield, 65%); Ms (m/z, %): 362 (M^+, 21.65%). IR (KBr). ν cm^{-1}: 3120 (CH, aromatic); 2985 (CH, aliphatic); 1675 (C=O); 1510 (C=C) 1400 (C-Cl). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): 1.00 (s, 3H, CH_3); 3.37 (s, 2H, CH_2); 4.53 (s, 1H, CH); 7.30 (s, 1H, NH); 7.50-8.02 (m, 5H, 4aromatic protons, NH); 12.85 (s, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-d_6), 9.35 (CH_3); 35.60 (C4); 51.96 (CH_2Cl); 105.90 (C12); 109.03 (C10); 128.23, 130.05, 131.22, 132.23, 138.25, 139.26 (aromatic carbons); 148.35 (C3); 158.02 (C11); 160.20 (C13); 164.23 (C7); 169.03 (C=O). Anal. Calcd. For $C_{16}H_{13}Cl_2N_5O$: C, 53.05%; H, 3.62%; Cl, 19.58%; N, 19.33%. Found: C, 52.93%; H, 3.50%; Cl, 19.40%; N, 19.01%.:

6-ethoxy-3-methyl-4-phenyl-4,8-dihydropyrazolo[3,4-b]pyrrolo[3,2-e]pyridin-5(1H)-one 7.

A solution of **2a** (0.01 mol) in Acetic anhydride (10 ml) and triethylorthoformate (1.45 gm, 0.01mol) was heated under reflux for 7 h. The solution was evaporated under vacuum and the residue was treated with ice water and was crystallized with proper solvent.

White crystals mp. 210-211 ºC, (EtOH). (Yield, 42%); Ms (m/z, %): 308 (M^+, 23%). IR (KBr). ν cm^{-1}: 3140 (CH, aromatic); 2988-2931 (C-H, aliphatic); 1738 (C=O); 1511 (C=C, aromatic). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): 1.23 (t, 3H, CH_3); 2.16 (s, 3H, CH_3); 4.30 (q, 2H, CH_2); 4.94 (s 1H, CH); 7.25-7.34 (m, 5H, aromatic protons); 8.61 (s, 1H, NH); 3.85 (s, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-d_6), 9.55 (CH_3, pyrazolo); 19.54 (CH_3); 35.60 (C4); 58.78 (CH_2); 105.03 (C9); 120.90 (C11); 127.23, 132.05, 131.22, 132.23, 138.25, 139.26 (aromatic carbons); 148.35 (C3); 159.02 (C10); 165.20 (C6); 169.23 (C12); 190.03 (C=O). Anal. Calcd. For $C_{17}H_{16}N_4O_2$: C, 66.22%; H, 5.23%; N, 18.17%. Found: C, 66.00%; H, 4.98%; N, 17.90%.

N'-(5-cyano-3-methyl-4-phenyl-4,7-dihydro-1H-pyrazolo[3,4-b]pyridin-6-yl)-N,N-dimethyli-midoformamide 8.

Solution of **2a** (0.01 mol) in Acetonitrile (10 ml) and dimethylformamid-dimethylacetal (1.19 g, 0.01mol) in a water bath for 9 hr. The precipitate was collected and the solution was evaporated under reduced vacuum and the residue was treated with ice water and crystallized.

White crystals mp. 270-271 ºC, Acetonitrile. (Yield, 63%); Ms (m/z, %): 306 (M^+); 230 (M^+-Ph, 100%). IR (KBr). ν cm^{-1}: 3100 (CH, aromatic); 2989-2950 (C-H, aliphatic); 1511 (C=C, aromatic). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): 1.79 (s, 3H, CH_3); 2.99 & 3.13 (2s, 6H,

$N(CH_3)_2$); 4.71 (s 1H, CH); 7.19-7.61 (m, 6H, aromatic protons and NH); 8.32 (s, 1H, CH); 12.14 (s, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-d_6), 9.35 (CH_3); 35.91 (C4); 45.30 & 47.21 (NMe_2); 79.96 (C5); 105.21 (C8); 121.63 (C≡N); 135.21, 142.36, 144.32, 146.32, 148.25, 156.32 (aromatic carbons); 159.52 (C3); 160.35 (C9); 166.52 (N=C-H); 172.33 (C6). Anal. Calcd. For $C_{17}H_{18}N_6$: C, 66.65%; H, 5.92%; N, 27.43%. Found: C, 66.45%; H, 5.72%; N, 27.23%.

Synthesis of Ethyl-3-methyl-4-(aryl)-6-phenyl-4,7-dihydro-1H-pyrazolo-[3,4-b]pyridine-5-carboxylate and 3-Methyl-4-(4-nitrophenyl)-6-phenyl-4,7-dihydro-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile 9a-e.

General Procedure: A solution of **1** (0.98 gm, 0.01 mol), arylaldehyde (0.01 mol) and 3-oxo-3-phenylpropanenitrile (1.45 gm, 0.01 mol) or ethyl benzoylacetate (1.92 g, 0.01 mol) in ethanol (10 ml) containing Ammonium acetate (0.98 gm, 2% excess) was heated under reflux for 4 hr. The solution was evaporated under vacuum and the solid remaining crystallized with the proper solvent.

Ethyl-3-methyl-4-(4-nitrophenyl)-6-phenyl-4,7-dihydro-1H-pyrazolo[3,4-b]pyridine-5-carbo-xylate ***9a.***

Pale yellow crystals mp. 276-277 ºC, (EtOH). (Yield, 81%); Ms (m/z, %): 404 (M^+, 42%). IR (KBr). ν cm^{-1}: 3410 (NH); 3210 (CH, aromatic); 2968-2702 (C-H, aliphatic); 1728 (C=O ester); 1513 (NO_2). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): 1.03-1.09 (t, 3H, CH_3); 1.91 (s, 3H, CH_3); 3.43-3.70 (q, 2H, CH_2); 4.98 (s, 1H, CH); 7.37-8.41 (m, 10H, 9 aromatic protons and NH); 12.15 (s, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-d_6), δ 9.48 (CH_3, pyrazolo); 15.78 (CH_3, ester); 31.69 (C4); 61.20 (CH_2, ester); 102.22 (C5); 109.91 (C8); 123.52, 124.21, 126.02, 127.23, 128.50, 128.96, 129.41, 131.65, 131.95, 134.52, 143.52, 143.21(aromatic carbons); 145.11 (C3); 157.08 (C9); 159.62 (C6); 168.21 (C=O). Anal. Calcd. For $C_{22}H_{20}N_4O_4$: C, 65.34%; H, 4.98%; N, 13.85%. Found: C, 65.14%; H, 4.79%; N, 13.65%.

Ethyl 4-(4-chlorophenyl)-3-methyl-6-phenyl-4,7-dihydro-1H-pyrazolo[3,4-b]pyridine-5-car-boxylate ***9b.***

White crystals mp. 196-197 ºC. (EtOH). (Yield, 71%); Ms (m/z, %): 393 (M^+, 16.32%). IR (KBr). ν cm^{-1}: 3232 (CH, aromatic); 2981 (CH, aliphatic); 1730 (C=O, ester); 1596 (C=C, aromatic). 0.78-0.83 (t, 3H, CH_3); 2.09 (s, 3H, CH_3); 3.79-3.94 (q, 2H, CH_2); 4.53 (s, 1H, CH); 7.20 (s, 1H, NH); 7.36-7.67 (m, 5H, 5aromatic protons); 7.90-8.00 (m, 4H, 4aromatic protons);12.10 (s, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-d_6), δ 9.48 (CH_3, pyrazolo); 16.78 (CH_3, ester); 32.79 (C4); 60.23 (CH_2, ester); 104.26 (C5); 110.81 (C8); 125.95, 126.59, 127.90, 128.55, 129.70, 130.23, 131.18, 132.30, 135.25, 139.17, 142.72, 144.63 (aromatic carbons); 145.11 (C3); 156.18 (C9); 160.62 (C6); 168.95 (C=O). Anal. Calcd. For $C_{22}H_{20}ClN_3O_2$: C, 67.09%; H, 5.12%; Cl, 9.00%; N, 10.67%. Found: C, 66.98%; H, 5.00%; Cl, 8.89%; N, 10.43%.

Ethyl-4-(4-dimethylaminophenyl)-3-methyl-6-phenyl-4,7-dihydro-1H-pyrazolo[3,4-b]pyridine-5-carboxylat ***9c.***

Yellow crystals mp. 220-221 ºC. (EtOH). (Yield, 35%); Ms (m/z, %): 402 (M^+, 19%). IR (KBr). ν cm^{-1}: 3168 (CH, aromatic); 2949 (CH, aliphatic); 1784 (C=O, ester); 1597 (C=C, aromatic). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): 1.19-1.24 (t, 3H, CH_3); 2.00 (s, 3H, CH_3); 2.74, 3.02 (2s, 6H, $2CH_3$); 4.15-4.22 (q, 2H, CH_2); 4.64 (s, 1H, CH); 6.50 (s, 1H, NH); 6.53-6.88 (m, 5H, 5aromatic protons); 7.88-8.00 (m, 4H, aromatic protons);11.22 (s, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-d_6), δ 9.35 (CH_3); 15.78 (CH_3, ester); 35.79 (C4); 44.32 & 45.44

(NMe$_2$); 61.23 (CH$_2$, ester); 101.26 (C5); 109.81 (C8); 113.87, 114.30, 125.59, 127.90, 128.55, 129.70, 131.18, 132.30, 135.25, 139.17, 142.72, 149.63 (aromatic carbons); 145.44 (C3); 157.18 (C9); 162.62 (C6); 169.26 (C=O). Anal. Calcd. For $C_{24}H_{26}N_4O_2$: C, 71.62%; H, 6.51%; N, 13.92%. Found: C, 71.42%; H,6.31%; N, 13.62%.

3-Methyl-4-(4-nitrophenyl)-6-phenyl-4,7-dihydro-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile ***9d.***
Red crystals mp. 280-281 ºC. (EtOH). (Yield, 76%); Ms (m/z, %): 357 (M$^+$, 1%). IR (KBr). ν cm^{-1}: 3420 (NH); 3190 (CH, aromatic); 2905 (CH$_3$); 2215 (C≡N); 1605 (NO$_2$). ^{1}H-NMR (270 MHz, DMSO-*d*$_6$, TMS): 2.02 (s, 3H, CH$_3$); 4.90 (s, 1H, CH); 7.29 (s, 1H, NH); 7.32-8.06 (m, 9H, aromatic protons); 11.37 (s, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-*d*$_6$), δ 9.45 (CH$_3$, pyrazolo); 33.79 (C4); 78.26 (C5); 101.81 (C8); 122.23 (CN); 123.57, 124.30, 126.59, 127.90, 128.55, 128.70, 131.18, 132.30, 134.25, 137.17, 139.72, 148.63 (aromatic carbons); 155.75 (C3); 158.18 (C9); 162.62 (C6). Anal. Calcd. For $C_{20}H_{15}N_5O_2$: C, 67.22%; H, 4.23%; N, 19.60%. Found: C, 67.01%; H, 4.05%; N, 19.46%.

4-[4-(dimethylamino)phenyl]-3-methyl-6-phenyl-4,7-dihydro-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile ***9e.***
Reddish crystals mp. 195-196 ºC. (EtOH). (Yield, 49%); Ms (m/z, %): 355 (M$^+$, 1%); 325 (M$^+$-2 CH$_3$, 1%). IR (KBr). ν cm^{-1}: 3167-3055 (C-H, aromatic); 2948-2794 (C-H, aliphatic); 2201(C≡N) 1500 (C=C, aromatic). ^{1}H-NMR (270 MHz, DMSO-*d*$_6$, TMS): 1.99 (s, 3H, CH$_3$); 2.43, 2.73 (2s, 6H, 2CH$_3$); 4.63 (s, 1H, CH); 6.53 (s, 1H, NH); 6.84-7.50 (m, 5H, Ph); 7.56-7.91 (m, 4H, 4aromatic protons) 11.37 (s, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-*d*$_6$), δ 9.42 (CH$_3$, pyrazolo); 32.79 (C4); 42.32 & 43.44 (NMe$_2$); 78.99 (C5); 104.81 (C8); 121.23 (CN); 111.87, 112.30, 118.59, 127.90, 128.55, 128.70, 131.18, 132.30, 134.25, 137.17, 139.72, 148.63 (aromatic carbons); 154.51 (C3); 156.18 (C9); 160.62 (C6). Anal. Calcd. For $C_{22}H_{21}N_5$: C, 74.34%; H, 5.96%; N, 19.70%. Found: C, 74.14%; H, 5.80%; N, 19.59%.

4-(2,5-dimethoxyphenyl)-3-methyl-6-phenyl-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile ***10.***
A solution of **1** (0.98 g, 0.01 mol), 2,5-dimethoxybenzaldehyde (0.01 mol) and 3-oxo-3-phenylpropanenitrile (1.45 g, 0.01 mol) in Ethanol (10 ml) containing piperdine (2ml) was heated under reflux for 4 hr. The clear solution was evaporated under vacuum and the residue was purified.

Yellow crystals mp. 350-351 ºC. (EtOH). (Yield, 62%); Ms (m/z, %): 373 (M$^+$). IR (KBr). ν cm^{-1}; 3420 (NH$_2$); 3104 (CH, aromatic); 2874 (C-H, Aliphatic); 2189 (C≡N), 1541 (C=C, aromatic).: ^{1}H-NMR (270 MHz, DMSO-*d*$_6$, TMS): 2.55 (s, 3H, CH$_3$); 3.83 (s, 3H, OCH$_3$); 3.89 (s, 3H, OCH$_3$); 5.95 (s, 1H, CH); 7.67-8.02 (m, 8H, aromatic protons); 9.01 (s, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-*d*$_6$), δ 9.45 (CH$_3$); 35.35 (C4); 56.16, 55.89 (2OCH$_3$); 76.86 (C5); 120.20 (C8); 122.53 (CN); 123.90, 126.031, 127.32, 128.21, 129.21, 130.21, 131.78, 134.32, 143.16, 145.23, 155.94, 156.42 (aromatic carbons); 153.13 (C9); 155.71 (C3); 168.18 (C6). Anal. Calcd. For $C_{22}H_{19}N_3O_3$: C, 70.76%; H, 5.13%; N, 11.25%. Found: C, 70.55%; H, 5.00%; N, 11.14%.

Ethyl-3,6-dimethyl-4-(4-nitrophenyl)-4,7-dihydro-1H-pyrazolo[3,4-b]pyridine-5-carboxylate ***11.***
A solution of **1** (0.98 gm, 0.01 mol), *p*-nitrobenzaldehyde (1.54 g, 0.01 mol) and ethylacetoacetate (1.29 gm, 0.01 mol) in ethanol (10 ml) containing Ammonium acetate (0.98 gm, 2% excess) was heated under reflux for 5 hr. The solution was evaporated under vacuum and the precipitate was purified.

Yellow crystals mp. 286-287ºC. (EtOH). (Yield, 68%); Ms (m/z, %): 342 (M^+, 5%). IR (KBr). ν cm^{-1}: 3150 (CH, aromatic); 2968-2702 (C-H, aliphatic); 1750 (C=O ester); 1516 (NO_2). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): 1.03 (t, 3H, CH_3); 2.10 (s, 3H, CH_3); 2.51 (s, 3H, CH_3); 3.35 (q, 2H, CH_2); 4.98 (s, 1H, CH); 7.37-8.37 (m, 5H, 4aromatic protons and NH); 11.39 (s, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-d_6), δ 9.59 (CH_3); 13.70 (CH_3, ester); 19.52 (CH_3, pyridine); 35.35 (C4); 65.95 (CH_2, ester); 104.25 (C5); 109.05 (C8); 126.031, 127.32, 130.21, 131.78, 143.85, 145.23 (aromatic carbons); 153.13 (C3); 156.71 (C6); 160.18 (C9); 168.85 (C=O). Anal. Calcd. For $C_{17}H_{18}N_4O_4$: C, 59.64%; H, 5.30%; N, 16.37%. Found: C, 59.34%; H, 5.21%; N, 16.21%.

Synthesis of Ethyl 6-amino-4-aryl-3-methyl-4,7-dihydro-1H-pyrazolo-[3,4-b]pyridine-5-carb -oxylate ***12a-c.***

General Procedure: A solution of **1** (0.98 g, 0.01 mol), arylaldehyde (0.01 mol) and ethycyanoacetate (1.13 gm, 0.01 mol) in ethanol (10 ml) containing Ammonium acetate (0.98 g, 2% excess) was heated under reflux for 5 hr. The solution was evaporated under vacuum and the residues were purified.

Ethyl 6-amino-4-(2,5-dimethoxyphenyl)-3-methyl-4,7-dihydro-1H-pyrazolo[3,4-b]pyridine-5-carboxylate ***12a.***

Yellow crystals mp. 201-202 ºC. (EtOH). (Yield, 54%); Ms (m/z, %): 358 (M^+, 60%). IR (KBr). ν cm^{-1}: 3353 (NH_2); 3120 (CH, aromatic); 2968-2702 (C-H, aliphatic); 1728 (C=O ester); 1510 (C=C, aromatic). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): 1.05 (t, 3H, CH_3); 2.07 (s, 3H, CH_3); 3.68 (s, 3H, OCH_3); 3.72 (s, 3H, OCH_3); 4.24 (q, 2H, CH_2); 4.95 (s, 1H, CH); 5.01 (s, 2H, NH_2); 6.76-7.20 (m, 4H, 3aromatic protons, NH); 11.36 (s, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-d_6), δ 9.35 (CH_3); 14.70 (CH_3, ester); 36.35 (C4); 56.20 & 57.32 ($2OCH_3$); 61.95 (CH_2, ester); 90.26 (C5); 109.05 (C8); 115.23, 119.51, 120.32, 126.21, 154.78, 155.23 (aromatic carbons); 144.71 (C3, pyrazolo); 155.18 (C9); 165.95 (C6); 168.85 (C=O). Anal. Calcd. For $C_{18}H_{22}N_4O_4$: C, 60.32%; H, 6.19%; N, 15.63%. Found: C, 60.13%; H, 6.02%; N, 15.42%.

Ethyl-6-amino-3-methyl-4-(4-nitrophenyl)-4,7-dihydro-1H-pyrazolo[3,4-b]pyridine-5-carb -oxylate ***12b.***

Pale yellow crystals mp. 150 ºC. (EtOH). (Yield, 30%); Ms (m/z, %):343 (M^+, 5%); 327 (M^+-NH_2, 1%). IR (KBr). ν cm^{-1}: 3495 (NH_2); 3150 (CH, aromatic); 2968-2702 (C-H, Aliphatic); 1740 (C=O); 1511 (C=C, aromatic). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): 2.35 (t, 3H, CH_3); 2.51 (s, 3H, CH_3); 4.32 (q, 2H, CH_2); 4.90 (s, 1H, CH); 7.84 (s, 2H, NH_2); 8.23-8.43 (m, 4H, 4aromatic protons); 8.57 (s, 1H, NH); 12.51 (s, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-d_6), δ 9.49 (CH_3); 13.70 (CH_3, ester); 35.35 (C4); 59.95 (CH_2, ester); 87.26 (C5); 105.05 (C8); 123.51, 124.32, 131.21, 132.78, 143.23, 145.42 (aromatic carbons); 147.71 (C3, pyrazolo); 159.18 (C8); 164.95 (C6); 168.85 (C=O). Anal. Calcd. For $C_{16}H_{17}N_5O_4$: C, 55.80%; H, 4.89%; N, 20.01%. Found: C, 55.97%; H, 4.99%; N, 20.40%.

Ethyl 6-amino-4-(4-chlorophenyl)-3-methyl-4,7-dihydro-1H-pyrazolo[3,4-b]pyridine-5-carb -oxylate ***12c.***

White crystals mp. 247-248 ºC. (EtOH). (Yield, 42%); Ms (m/z, %): 332 (M^+, 3%). IR (KBr). ν cm^{-1}: 3659-3409 (NH_2); 3174 (CH, aromatic); 2968 (C-H, Aliphatic); 1750 (C=O); 1511 (C=C, aromatic). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): 1.06 (t, 3H, CH_3); 1.79 (s, 3H, CH_3); 4.35 (q, 2H, CH_2); 4.64 (s, 1H, CH); 6.93 (s, 2H, NH_2); 7.18-7.39 (m, 5H, 4aromatic protons & NH); 12.14 (s, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-d_6), δ 9.29 (CH_3); 13.70

(CH_3, ester); 35.35 (C4); 60.95 (CH_2, ester); 87.26 (C5); 105.05 (C8); 128.031, 129.32, 131.21, 132.78, 138.85, 142.23 (aromatic carbons); 146.71 (C3, pyrazolo); 156.18 (C9); 163.95 (C6); 168.85 (C=O). Anal. Calcd. For $C_{16}H_{17}ClN_4O_2$. C, 57.75%; H, 5.15%; Cl, 10.65%; N, 16.84%. Found: C, 57.35%; H, 5.00%; Cl, 10.23%; N, 16.64%.

Ethyl 3-methyl-4-(4-nitrophenyl)-6-oxo-4,5,7-trihydro-1H-pyrazolo-[3,4-b]pyridine-5-carb -oxylate ***13.***

A solution of **1** (0.98 g, 0.01 mol), *p*-nitrobenzaldehyde (1.54 g, 0.01 mol) and diethylmalonate (1.61 g, 0.01 mol) in ethanol (10 ml) containing Ammonium acetate (0.98 gm, 2% excess) was heated under reflux for 5 h. The solution was evaporated under vacuum and the residues were purified.

Reddish crystals mp. 265-266 ºC. (EtOH). (Yield, 63%); Ms (m/z, %): 344 (M^+, 60%). IR (KBr). ν cm^{-1}: 3050 (CH, aromatic); 2969-2857 (CH, aliphatic); 1731 (C=O, ester); 1685 (C=O); 1512 (C=C, aromatic). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): 1.13-1.16 (t, 3H, CH_3); 1.87 (s, 3H, CH_3); 3.31-3.43 (q, 2H, CH_2); 4.07 (s, 1H, CH); 4.93 (s, 1H, CH); 7.32-8.01(m, 4H, aromatic), 8.10 (s, 1H, NH), 11.3 (s, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-d_6), δ 9.39 (CH_3, pyrazolo); 13.55 (CH_3' ester); 32.35 (C4); 62.95 (CH_2, ester); 65.52 (C5); 105.05 (C8); 127.03, 128.32, 131.21, 132.78, 147.85, 149.23 (aromatic carbons); 143.13 (C3, pyrazolo); 157.18 (C9); 168.85 (C=O); 173.21 (C6). Anal. Calcd. For $C_{16}H_{16}N_4O_5$: C, 55.81%; H, 4.68%; N, 16.27%. Found: C, 55.60%; H, 4.48%; N, 16.00%.

1-[4-(2,5-dimethoxyphenyl)-3,6-dimethyl-4,5-dihydro-1H-pyrazolo[3,4-b]pyridin-5-yl]-ethan -one ***14.***

A solution of **1** (0.98 g, 0.01 mol), 2,5-dimethoxybenzaldehyde (1.66 g, 0.01 mol) and acetylacetone (1.00 g, 0.01 mol) in ethanol (10 ml) containing Ammonium acetate (0.98 gm, 2% excess) was heated under reflux for 5 h. The solution was evaporated under vacuum and the solid collected was crystallized with the proper solvent.

Dark white crystals mp. 260-261 ºC. (EtOH). (Yield, 85%); Ms (m/z, %): 327 (M^+, 16%). IR (KBr). ν cm^{-1}: 3410 (NH); 3150 (CH, aromatic); 2969-2857 (CH_3); 1750 (C=O); 1512 (C=C, aromatic). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): 1.08 (s, 3H, CH_3); 2.05 (s, 3H, CH_3); 3.25 (s, 1H, CH); 3.41 (s, 3H, -CH_3); 3.63 (s, 3H, OCH_3); 3.73 (s, 3H, OCH_3); 5.02 (s, 1H, CH); 6.64-6.80 (m, 3H, aromatic), 11.12 (s, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-d_6), δ 9.49 (CH_3); 19.52 (CH_3); 25.32 (CH_3, acetone), 35.42 (C4); 60.30, 62.32 (2OCH_3); 105.35 (C8); 119.05 (C5); 121.31, 122.32, 125.21, 131.78, 153.85, 155.23 (aromatic carbons); 144.18 (C3); 151.13 (C6); 160.71 (C9); 170.10 (C=O). Anal. Calcd. For $C_{18}H_{21}N_3O_3$: C, 66.04%; H, 6.47%; N, 12.84%. Found: C, 65.95% H, 6.30%; N, 12.64%.

Bioassay

Cytotoxic effect on human cell line (HEPG 2)

Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan *(Mosmann)* [19]. Procedure: All the following procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Sanford, ME, USA). The method was carried out according to Thabrew20. Cells were batch cultured for 10 days, then seeded at concentration of 10x103 cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37 ºC for 24 hr under 5% CO_2 using a water jacketed Carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium (without serum) was added and cells were incubated either alone (negative control) or with

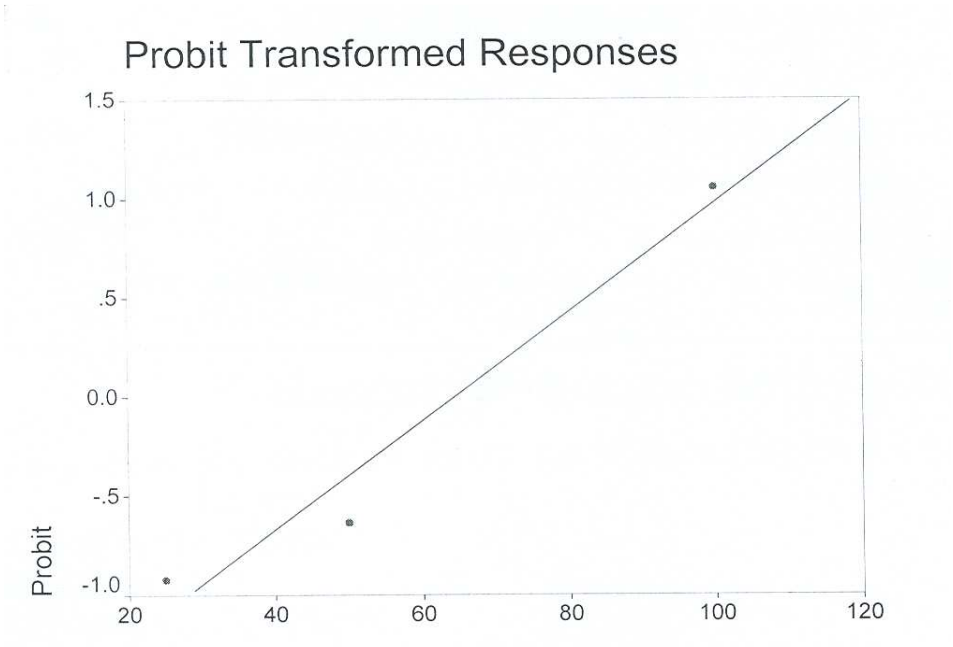
different concentrations of sample to give a final concentration of (100-µg/ml). Cells were suspended in RPMI 1640 medium [for HePG2] , 1% antibiotic-antimycotic mixture (10,000 µg/ml Potassium Penicillin, 10,000 µg/ml Streptomycin Sulfate and 25 µg/ml Amphotericin B) and 1% L-glutamine in 96-well flat bottom microplate at 37 ºC under 5% CO_2. After 48 h of incubation, medium was aspirated, 40 ul MTT salt (2.5 µg/ml) were added to each well and incubated for further four hours at 37ºC under 5% CO_2. To stop the reaction and dissolving the formed crystals, 200 µL of 10% Sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37 ºC. A positive control which composed of 100µg/ml of Annona cherimolia extract was used as a known cytotoxic natural agent which gives 100% lethality under the same conditions.

The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595nm and a reference wavelength of 620nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent t-test by SPSS 11 program. DMSO is the vehicle used for dissolution of plant extracts and its final concentration on the cells was less than 0.2%.

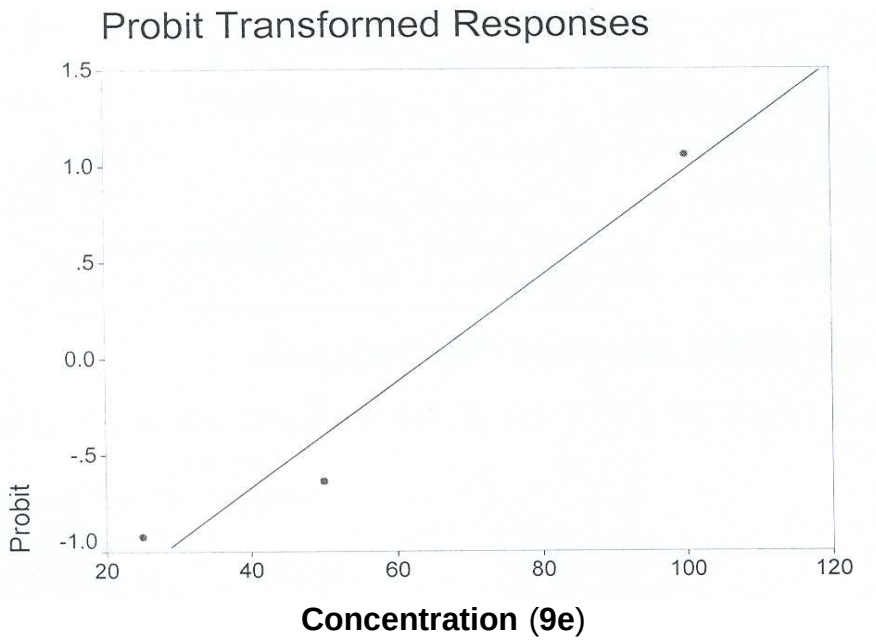
The percentage of change in viability was calculated according to the formula:

((Reading of extract / Reading of negative control) -1) x 100

A probit analysis was carried for LC_{50} determination using SPSS 11 program



Concentration (12c)



Conclusion

Ten compounds were introduced into the cytotoxcity bioassay on Human tumor cell line (HEPG2). The results indicated that compounds **9e** and **12c** are the most active cytotoxic agents in the tested compounds. They revealed LC_{50} values 66.6 and 78.4, respectively. The other tested compounds **12b** and **4b** ranged between 37.7 and 49% at 100 ppm, while compound **2c**, **9c**, **4a**, ranged between 52.3% and 70% at 100ppm (weak activity). On the other hand, there is no cytotoxcity effect for compounds **11** and **2c** on the (HEPG2) cell line.

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